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Section B.—Biological Sciences.

On Opsonins in Relation to Red Blood-cells.

By J. O. Wakelin Barratt, M.D., D.Sc. Lond.

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(From the Lister Institute of Preventive Medicine.)

In a previous communication[*] it was pointed out that opsonins, whose existence in relation to bacteria is generally recognised, are also produced by injecting foreign red blood-cells into the peritoneal cavity. The latter variety of opsonin, which is thermostable, not being destroyed by exposure to 58° C. for 30 minutes, is taken up by red blood-cells of the kind used for injection. Such red blood-cells, after being thus "sensibilised" by opsonin, are ingested by leucocytes.

Since it appeared obvious that the occurrence of phagocytosis might, under suitable conditions of experiment, be made use of in order to discover if a serum were capable of sensibilising red blood-cells, that is, if it contained opsonin corresponding to the red blood-cells employed, it was determined to ascertain if, proceeding along the line of investigation thus suggested, a method for the estimation of red blood-cell opsonins could be devised. It was further decided to study the nature of the action of such opsonins upon red blood-cells.

Estimation of Opsonin.

In attempting to make use of phagocytosis for detecting the presence of opsonin in serum a serious difficulty is at the outset encountered owing to the occurrence of spontaneous phagocytosis. When the red blood-cells of the rabbit, for example, are injected into the guinea-pig, opsonin for such red blood-cells develops in the latter's serum, which (after destruction of complement (alexin) by heating for 20 to 30 minutes at 58° C.*) may now be used to sensibilise the red blood-cells of the rabbit. If the sensibilised red blood-cells are added to the leucocytes of the guninea-pig, contained in the (heated) normal serum of the guinea-pig, extremely active phagocytosis of the red blood-cells at once takes place. If, however, sensibilisation is omitted, phagocytosis is still observed, though in considerably diminished degree. Attempts to overcome the disturbing effect of this residual or "spontaneous" phagocytosis have not as yet met with a sufficient degree of success to enable estimations of opsonin to be carried out in such cases.

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[*] J. O. W. Barratt, "The Phagocytosis of Red Blood-cells," 'Roy. Soc. Proc.,' B, 1905, vol. 76, p. 524.

There is, however, another class of cases in which spontaneous phagocytosis does not occur. Leucocytes contained in the serum natural to them do not ingest the red blood-cells with which they are normally associated.† If, for instance, the red blood-cells of the guinea-pig are injected into the abdominal cavity of the rabbit and the serum of the latter is employed for sensibilisation, the leucocytes of the guinea-pig, contained in (inactivated) guinea-pig serum are now seen to ingest such sensibilised red cells, though if sensibilisation is omitted no phagocytosis whatever occurs.

A serum of this kind was prepared from a rabbit, which will henceforth be referred to as Rabbit A. The serum was used for the series of experiments recorded in the tables.

Rabbit A.—Weight, 2300 grammes. Intraperitoneal injections were made as follows:—

On the 1st day the red blood-cells of 7·5 c.c. of guinea-pig's blood.

On the 11th day the red blood-cells of 4·5 c.c. of guinea-pig's blood.

On the 21st day the red blood-cells of 4·5 c.c. of guinea-pig's blood.

On the 118th day the rabbit was bled to death. The serum obtained was inactivated by heating to 58° C. for 30 minutes.

An attempt was first made to ascertain if opsonin could be removed from the serum of Rabbit A by the washed red blood-cells of the guinea-pig, added in varying amounts and acting upon the serum (diluted and undiluted) for periods of time ranging from five minutes to five hours (Table I). During this time the mixture of serum and red cells was continually shaken, either by hand or in a motor-driven shaker. The mixture was then centrifugalised and the supernatant liquid pipetted off (great care being taken to ensure the complete removal of the red blood-cells).

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* Otherwise hæmolysis of the red blood-cells occurs.

† All the experiments with sensibilised red blood-cells, given in the tables, were accompanied with control experiments on normal red cells. In these, as in numerous other control experiments not referred to here, the results as regards phagocytosis were invariably negative.

The washed red blood-cells of 0·005 c.c. of guinea-pig's blood were next added to 1 c.c. of the deopsinated serum, and the whole agitated for two hours at 37° C. The sensibilised red cells, after being washed in 0·85 per cent. sodium chloride solution, were added in excess to the leucocytes of the guinea-pig in inactivated guinea-pig serum and kept on a warm stage (37° C.) for two hours. The leucocytes were then examined under the microscope and the percentage containing red blood-cells noted, 2000 to 4000 leucocytes being enumerated. The percentages thus obtained are only approximately correct,* as will be readily understood by reference to Table III, but by making a number of tests under conditions as nearly as possible identical in respect of every detail, fairly concordant results are obtained.

The results, given in Table I, showed that it was possible to remove opsonin from the serum employed, provided that a sufficient number of red cells were used. After the red blood-cells of one or more parts of guinea-pig's blood had acted upon two parts of the serum of Rabbit A, the serum had completely lost its power of sensibilising red cells; in other words, it no longer contained opsonin in recognisable amount. If the red blood-cells of one part of guinea-pig's blood were shaken with four parts of serum, sufficient opsonin was usually left in the serum to sensibilise red cells; this was especially the case when the period for deopsination was short. In Table I no indication of the degree to which opsonin is removed is afforded beyond the fact that a period of five minutes is too short to effect complete removal at 37° C.

The enquiry now presents itself, how much serum is required to sensibilise the red blood-cells of a given volume of guinea-pig's blood? To investigate this point the series of observations recorded in Table II was undertaken.

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* The degree of phagocytosis obtained, apart from the degree to which sensibilisation of the red cells has been carried, depends chiefly upon the uniformity and the closeness of distribution of leucocytes and red cells. To secure the maximum degree of phagocytosis the cells must be evenly distributed and the leucocytes must not be separated by more than a few cell breadths from the erythrocytes, for leucocytes do not appear to recognise the presence of the latter until their processes come in contact with them. Difficulty was occasionally experienced owing to the occurrence of some degree of agglutination of the red cells after treatment with the rabbit's serum; separation could usually be effected by shaking, provided the proportion of serum employed was not too great (*i.e.*, $\frac{B}{S}$ not above $\frac{1}{20}$, compare Table III). The condition of the leucocytes also affects the degree to which the red cells are taken up. If a sufficiently extended series of tests are made the condition of the leucocytes tends to become the same in each experiment.

Table I.—Deopsination of Serum of Rabbit A, by means of the Red Blood-cells the Guinea-pig. Temperature of Experiment, 37° to 38° C.

No. of expt.	Dilution of serum.	$\frac{B^*}{S}$.	Phagocytosis.† [The duration of the deopsinating action of the red blood-cells on the serum is given in brackets.]									
			[5 min.].	[10 min.].	[20 min.].	[40 min.].	[1 hr.].	[$1\frac{1}{3}$ hr.].	[2 hr.].	[3 hr.].	[4 hr.].	[5 hr.].
1	1 in 8	$\frac{8}{1}$							—			
2	Undiluted	$\frac{4}{1}$							—			
3	1 in 8	$\frac{2}{1}$							—			
4	Undiluted	$\frac{1}{1}$							—			
5	”	$\frac{1}{1 \cdot 6}$				—	—		—			—
6	1 in 8	$\frac{1}{2}$							—			
7	1 in 2	”			—		—				—	—
8	1 in 8	$\frac{1}{4}$							—			
9	Undiluted	”							—			
10	”	”	+ + +		+ +			—		—		
11	1 in 8	”	+ + +		+ + +			—		—		
12	Undiluted	”	+ + +		+ + +			+ + +		+		
13	1 in 8	”	+ + +		+ +			+ +		+		
14	Undiluted	”	+ + +		—			—		—		
15	1 in 8	”	+ + +		+ + +			+ + +		+ + +		
16	Undiluted	”	+ +		+ +			+		+		
17	1 in 8	”	+ + +		—			+ + +		+ + +		
18	Undiluted	”			—			+ +		+ +		
19	1 in 8	”			—			—			—	
20	”	”	+ + +	+ + +	+ + +	+ + +						
21	”	”			+ +	+ +						

* The washed red blood-cells of guinea-pig's blood (B) were added to the serum of rabbit A (S) in the proportions given. Thus, $\frac{B}{S} = \frac{8}{1}$ means that eight parts of guinea-pig's blood and one part of the serum of the rabbit were taken.

† In order to test phagocytosis, the red blood-cells of 0·005 c.c. of guinea-pig's blood were sensibilised with 1 c.c. of the deopsinated serum, diluted or undiluted, and then added to leucocytes obtained from the guinea-pig. The sign — means that no phagocytosis occurred; + that 0·2 per cent. to 0·7 per cent. of the leucocytes contained red blood-cells; + + that 1 per cent. to 1·5 per cent. contained red cells; + + + that the maximum degree of phagocytosis was obtained (2 per cent. to 5 per cent., in one instance 10 per cent., in another 20 per cent.).

In these experiments the red blood-cells of gradually diminishing amounts of guinea pig's blood were added to a given volume of the serum of Rabbit A, and the whole shaken for two hours at 37° C., the red cells being then washed and tested as to their power of exciting phagocytosis. It will be seen that so long as the ratio of the former to the latter exceeds 3 : 1 the red blood-cells are quite insufficiently sensibilised and fail to excite phagocytosis when added to the leucocytes of the guinea-pig. When the proportion is 1 : 1, phagocytosis becomes marked,* and no further increase in the amount of serum employed causes any recognisable augmentation of the degree of phagocytosis obtainable. From this it may be concluded that, as far as phagocytosis is concerned, this ratio represents complete sensibilisation. This does not, however, represent the maximum amount of opsonin which can be taken up, for it appears, from Table I, that the red blood-cells of one part of guinea-pig's blood in some of the experiments removed opsonin to such an extent from four parts of serum that the serum was no longer capable of sensibilising red blood-cells, though in other experiments sufficient opsonin for this purpose was still left. An explanation of the difference in the amount of opsonin taken up in the two cases is probably to be found in the circumstances that phagocytosis is essentially a surface phenomenon depending upon the degree to which the outer part of the red blood-cell is sensibilised, and no advantage arises from the opsonin acting upon the cell in its whole thickness.

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* The highest degree of phagocytosis obtainable was found to be that in which 2 to 6 per cent. of the leucocytes had taken up red cells at the end of 2 hours at 37° C. (after 2 hours little or no increase occurred). This range represents the variation to be expected under the most favourable circumstances. Percentages of 20, 12 and 10 were occasionally obtained, but were quite exceptional.

A striking fact becomes evident from Table II, namely that dilution of the serum does not arrest the process of sensibilisation of red blood-cells. When the red cells of one part of guinea-pig blood are mixed with one part (or more) of serum, sensibilisation occurs and phagocytosis results equally whether the serum is undiluted or is mixed with as much as 500 parts of 0·85-per-cent. sodium chloride solution.

The ratio noted above $\left(\frac{B}{S} = \frac{1}{1}\right)$ as representing the minimum relative amount of serum necessary for the production of marked phagocytosis enables an idea to be formed of the delicacy of the test of deopsination employed in Table I. If the red blood-cells of 0·005 c.c. of the blood of the guinea-pig are mixed with 1 c.c. of deopsinated serum, and are found to become so far sensibilised that marked phagocytosis is obtained, it follows that the serum contains, after deopsination, at least 0·5 per cent. of the amount of opsonin originally present. If, however, phagocytosis is found, on repeated testing, to be always feeble or to be absent, it follows that less than this percentage is present. By adding the red blood-cells of varying amounts of guinea-pig's blood to 1 c.c. of the serum, and noting the point at which sensibilisation becomes incomplete and phagocytosis feeble or absent—and, as is illustrated in Table II, this point is indicated with a fair amount of clearness if a sufficient number of experiments are made, the same technique being closely followed in each—it appeared to be feasible to ascertain approximately the degree to which opsonin had been removed from the serum in question.

Influenced by this line of thought, the series of determinations in Table III, which forms a continuation of Experiments 8 to 21 in Table I,

Table II.—Experiments to Determine the Degree to which Red Blood-cells must be
Rabbit A, and the Red Blood-cells

No. of experiment.	Duration of action of red blood-cells on serum.	At temperature of—	Phagocytosis* (the dilution						
			$\frac{B^*}{S} = \frac{32}{1}$.	$\frac{16}{1}$.	$\frac{8}{1}$.	$\frac{4}{1}$.	$\frac{3}{1}$.	$\frac{2}{1}$.	$\frac{1·5}{1}$.
1	2 hours.	37° C.							— (1 in 256)
2	"	"							
3	"	"							
4	"	"							
5	"	"							+ (1 in 512)
6	"	"							
6	"	"							
6	"	"							
6	"	"							
7	"	"	— (1 in 14)	— (1 in 7)	— (1 in 7)		+ + (1 in 7)		
8	"	"			— (1 in 10)	— (1 in 10)		+ (1 in 7)	
9	"	"				— (1 in 10)		— (1 in 10)	
10	"	"				— (1 in 100)		+ + (1 in 100)	
11	"	"							

* Phagocytosis was tested by sensibilising the washed red blood-cells of guinea-pig's blood (B) with the serum
the rabbit's serum were taken. After sensibilisation the red cells were washed in 85-per-cent. sodium
0·2 to 0·6 per cent. of the leucocytes ingested red blood-cells; + + that 0·8 to 1·3 per cent. contained
instance 9 per cent., in another 10 per cent.).

was carried out. Portions of the serum of Rabbit A, diluted with an equal volume of 0·85-per-cent. sodium chloride solution, were mixed with the washed red cells of guinea-pig blood in the proportion of 4 : 1, for periods of time varying from 5 minutes to 3 hours and 20 minutes, and subsequently tested for the presence of opsonin, as in previous experiments (A to D, Table III). A second (E to H), and third (I to M) series of the same character were also carried out, the serum being diluted to 1 in 8 and 1 in 7 respectively. The tests of phagocytosis were made in groups, sometimes with diminishing amounts of the same serum (arranged horizontally in Table III), as in Experiments 1 to 18, sometimes with serum deopsinated for different periods of time (these experiments are marked with an asterisk sensibilised in order to excite Phagocytosis. The Serum employed was that of were those of the Guinea-pig.

of the serum is given in brackets).

$\frac{1}{1}$.	$\frac{1}{1\cdot5}$.	$\frac{1}{2}$.	$\frac{1}{3}$.	$\frac{1}{4}$.	$\frac{1}{6}$.	$\frac{1}{8}$.	$\frac{1}{12}$.	$\frac{1}{24}$.
	+ + + (1 in 128)		+ + + (1 in 64)		+ + + (1 in 32)		+ + + (1 in 16)	+ + + (1 in 8)
	+ (1 in 512)		+ (1 in 256)		+ (1 in 128)		+ (1 in 64)	+ + (1 in 32)
							+ + + (1 in 16)	+ (1 in 16)
	+ + (1 in 128)		+ + + (1 in 64)		+ + + (1 in 32)			
	+ + + (1 in 256)		+ + + (1 in 128)		+ + + (1 in 64)			
+ + + (1 in 8)								
+ + + (1 in 16)								
+ + + (1 in 32)								
+ + + (1 in 512)								
+ + + (1 in 5)								
+ (1 in 10)		+ (1 in 10)		+ (1 in 10)		+ (1 in 10)		
+ + + (1 in 100)		+ + + (1 in 100)		+ + (1 in 100)		+ + + (1 in 100)		
						+ + + (1 in 100)		

of Rabbit A in the proportions given. Thus, $\frac{B}{S} = \frac{32}{1}$ means that 32 parts of guinea-pig's blood and 1 part of chloride solution and added to the leucocytes. The sign — means that no phagocytosis was observed; + that red cells; and + + + that the maximum degree of phagocytosis was reached (2 to 6 per cent., in one

and are arranged vertically in Table III) as in Experiments 19 to 30. In spite of the variations exhibited by individual experiments fairly definite limiting values are obtainable for most of the sera. Thus in the experiments with Serum A a sharp diminution of sensibilising power is seen when the ratio B/S is less than 1/25, whence it is concluded that 25 parts of serum after deopsination contain only as much opsonin as one part originally did, that is to say, the opsonin has been reduced to 4 per cent. of the amount present at the beginning of the experiment; with B and C a distinct but less marked change in the sensibilising power of the sera is seen, indicating reduction of opsonin to 2 per cent. and 1 per cent. respectively of the amount originally present; the opsonin in D is obviously reduced to less than

Table III.—Exhibiting the Degree of Deopsination of the Serum of Rabbit A, effected by the Red Blood-cells of the Guinea-pig acting for varying Periods of Time. Deopsination was effected by adding the washed Red Blood-cells of one part of Guinea-pig Blood to four parts of the Rabbit's Serum.

No. of experiment.	Serum.	Dilution of serum.	Time allowed for deopsination.	Temperature at which deopsination was effected.	Phagocytosis of red blood-cells of the guinea-pig, sensibilised by the deopsinated serum of Rabbit A. In carrying out these tests, the washed red blood-cells of one part of guinea-pig's blood were added to the deopsinated serum in the following amounts:—																						Approximate amount of opsonin still remaining, the amount originally present being taken at 100.
					$\frac{B}{S} = \frac{1}{500}$	$\frac{1}{200}$.	$\frac{1}{100}$.			$\frac{1}{66}$.		$\frac{1}{50}$.			$\frac{1}{32}$.	$\frac{1}{25}$.			$\frac{1}{16}$.	$\frac{1}{12}$.	$\frac{1}{8}$.	$\frac{1}{6}$.	$\frac{1}{4}$.	$\frac{1}{3}$.	$\frac{1}{2}$.	$\frac{1}{1}$.	
			h. m.		p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	p. c.	per cent.
1	A	1 in 2	0 5	37° C.	4*			2*	20*						7				0		0		0		0	0	
2	,,	,,	,,	,,								12				10				0·3		0·2					4
	B	,,	0 20	,,	0*	0*	1*	3*	1*																		
3	,,	,,	,,	,,								2				0·5				0·5		0					2
	C	,,	1 20	,,	0*	1*	1·5*	2*	0*																		
4	,,	,,	,,	,,				2		0		0															1
5	D	,,	3 20	,,	0*	1*	0·4*	0·5*	0*	0					0				0		0		0		0	0	
6	,,	,,	,,	,,												0·5											< 1
7	E	1 in 8	0 5	,,						10	2*				6	10*	2*										
8	,,	,,	,,	,,																							
9	F	,,	0 20	,,						1·5	2*			0*	1	5*	1·5*	2*		6		10		0			15
10	,,	,,	,,	,,																							
	G	,,	1 20	,,							4*			0*		0*	1*	1*				0·5		0			4
11	,,	,,	,,	,,																							
	H	,,	3 20	,,							2*			0*		0*	0·7*	0*		0·7		0					1·5
																											< 1·5
12	I	1 in 7	0 5	,,						5		10*	0*		8												
13	,,	,,	,,	,,																							
14	J	,,	0 10	,,						6		6*	0*		1						4		0·7		0	0	12
15	,,	,,	,,	,,																							
16	K	,,	0 20	,,						2		5*	+*		3						1·4		0		0	0	> 2
17	L	,,	0 40	,,								4*	+*		0	0			0		0		0		0	0	> 3
18	M	,,	4 0	34°·5 C.								0*	0*		0	0			0		0		0		0	0	2
			No of experiment......		19	20	21	22	23		24	25	26	27		28	29	30									

The low percentages have not the same value as the high percentages, since any imperfection of technique, in particular such as would lead to lowering of vitality of the leucocytes, or would result in defective admixture of the cells, causes diminution of the percentage noted. Hence when experiments are repeated with the same serum, the higher percentages are the more accurate. The low percentages often obtained when the amount of serum employed is relatively high $\left(\frac{B}{S} = \frac{1}{100} \text{ to } \frac{1}{500}\right)$ are due to the occurrence of agglutination, which in such cases causes difficulty, especially if the red cells have been too strongly centrifugalised.

1 per cent. of its original value, but beyond this its titre cannot be further defined from the experiments made; and similarly with the remaining sera.

The experiments in Table III show that a very rapid removal of opsonin takes place during the first five minutes, and that subsequently the removal of opsonin becomes considerably slower, some of the opsonin originally present still remaining in Sera D and H even at the end of several hours. As would be expected, the removal rate is seen to be more rapid with the more concentrated serum (A to D) than in the more dilute serum (E to H, and I to M).

The experiments in Table III show that, when spontaneous phagocytosis is avoided, the estimation of red blood-cell opsonin may be carried out. Sera from different sources may be compared as to their opsonic content by determining the minimum amount of serum required to sensibilise a given bulk of red cells so as to produce the maximum degree of phagocytosis. An alternative method is to determine the minimal bulk of red blood-cells required to deopsinate unit of volume of the sera.

The Interaction of Red Blood-cell and Opsonin.

It is not possible from the data above given to arrive at a definite conclusion as to the nature of the process occurring when red blood-cells are sensibilised by opsonin.

It has been already pointed out that even considerable dilution of the serum of Rabbit A did not abolish sensibilisation so long as the ratio $\frac{B}{S} = 1$ was preserved. This circumstance is readily explicable on the assumption that a chemical reaction takes place, but it is also equally well explicable on the assumption that a physical process of adsorption or selective solubility occurs. The relation between concentration and adsorption has been determined by Ostwald* to be

$$C_1 = aC_2^n,$$

where C_1 is the concentration of the adsorbed substance, C_2 that of the substance in solution, and a and n are constants. By suitably choosing the values of a and n, the value of C_1 could be left practically unchanged when C_2 was reduced to 1/500 of its initial value.† So far as the behaviour of opsonins in respect of dilution has up to the present been investigated,

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* 'Lehrbuch d. allg. Chemie,' 1891, vol. 1, 2te Auflage, p. 1096.

† For example, if $a = 1{,}000{,}000$ and $n = 1$, in which case (since $\frac{C_1}{C_2} = \frac{1000000}{1}$) all but 1×10^{-6} of the opsonin originally present in the liquid would pass to the red blood-cells. If n were less than 1, the proportion taken up by the red cells would be still greater. Unfortunately the estimation of very small percentages of opsonin requires much larger amounts of serum than can be obtained from rabbits.

Table IV.—The Character of the Interaction of Erythrocytal Opsonins with red Blood-cells. Temperature of Experiment 37° C. (Compare Table III.)

Experiments.	Dilution of serum.	Value of—				Relative value of—				Period.	Values of k, calculated from the formulæ—			
		t_0.	t_1.	t_2.	t_3.	C_0.	C_1.	C_2.	C_3.		$\frac{1}{t''-t'}\log\frac{C'}{C''}$.	$\frac{1}{t''-t'}\left(\frac{1}{C''}-\frac{1}{C'}\right)$.		$\frac{1}{t''-t'}\left(\frac{1}{[C'']^2}-\frac{1}{[C']^2}\right)$.
A to D, Table III	1 in 2	0′	5′	20′	80′	100	4	2	1	t_0 to t_1	0·0219	0·0025	mean 0·0016	0·0017
										t_0 to t_2	0·0085	0·0025		0·0125
										t_0 to t_3	0·0025	0·0012		0·0063
										t_1 to t_2	0·0020	0·0017		0·0125
										t_1 to t_3	0·0012	0·0012		0·0062
										t_2 to t_3	0·0005	0·0008		0·0125
E to H, Table III	1 in 8	0′	5′	20′	80′	100	15	4	1·5	t_0 to t_1	0·0165	0·0011	mean 0·0010	0·0005
										t_0 to t_2	0·0699	0·0012		0·0016
										t_0 to t_3	0·0022	0·0008		0·0028
										t_0 to t_2	0·0028	0·0012		0·0020
										t_1 to t_3	0·0013	0·0008		0·0030
										t_2 to t_3	0·0007	0·0007		0·0032
I to M, TableIII	1 in 7	0′	5′	40′	—	100	12	2	—	t_0 to t_1	0·0186	0·0025	mean 0·0016	0·0069
										t_0 to t_2	0·0425	0·0012		0·0033
										t_1 to t_2	0·0020	0·0012		0·0035

the results obtained do not permit a conception of the nature of the interaction of red cell and opsonin to be formed.

In a stoichiometric reaction which proceeds slowly, not only is it possible to establish the molecular character of the reaction by means of determinations of the reaction rate, but the degree of the reaction may also be ascertained. The general formulæ for stoichiometrical reactions are

$$t''-t' = k \log C'/C''$$

for monomolecular reactions, and

$$t''-t' = k\,[1/(C'')^n - 1/(C')^n]$$

for multimolecular reactions, where C' is the concentration at time t', C'' that at time t'', n is unity for bimolecular reactions, and k is a constant. The truth of these formulæ has been established for homogeneous systems. Whether they are applicable to heterogeneous systems as in the interaction of toxin and antitoxin has been called in question. In the case of the heterogeneous system formed by red blood-cell + opsonin, the data above given enable the applicability of the formulæ in question to be tested empirically. In Table IV, from the data furnished by Table III which are repeated, the values of k are calculated: (1) as for a monomolecular reaction; (2) for $n = 1$; and (3) for $n = 2$.* The values of k for the first and third formulæ exhibit wide variations; the values for $n = 1$ show a fair correspondence, the average of all the experiments being 0·0014, while the highest and lowest values are 0·0008 and 0·0025 respectively. Thus the striking result is obtained that in the heterogeneous system under consideration a relation is exhibited similar to that observed in a bimolecular stoichiometric reaction occurring in a homogeneous system.

Summary.

It has been shown that—

1. By employing phagocytosis as a test of the presence of red blood-cell opsonin, and avoiding spontaneous phagocytosis by suitable conditions of experiment, quantitative determinations of the opsonic content of serum may be made.

2. In the experiments made, the interaction of opsonin and red blood-cell proceeded at a rate corresponding to that exhibited in a bimolecular stoichiometric reaction.

In conclusion, I must express my indebtedness to the assistance I have received from Dr. Allan Macfadyen, in whose laboratory this research was conducted, and from Mr. J. A. Craw and Professor Donnan.

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[*] The concentrations of red blood-cell and of opsonin are regarded as equivalent, since the opsonin is present in the minimum proportion necessary to lead to full sensibilisation of the red blood-cells.